Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2927–2930
Bioorganic & Medicinal Chemistry Letters

Selective and dual action orally active inhibitors of thrombin and factor Xa

Robert J. Young,[a,*] David Brown,[a] Cynthia L. Burns-Kurtis,[b] Chuen Chan,[a] Máire A. Convery,[a] Julia A. Hubbard,[a] Henry A. Kelly,[a] Anthony J. Pateman,[a] Angela Patikis,[a] Stefan Senger,[a] Gita P. Shah,[a] John R. Toomey,[b] Nigel S. Watson[a] and Ping Zhou[a]

[a]*GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, UK*
[b]*GlaxoSmithKline, 709 Swedeland Road, King of Prussia, PA 19406, USA*

Received 23 February 2007; revised 23 March 2007; accepted 24 March 2007
Available online 30 March 2007

Abstract—The synthetic entry to new classes of dual fXa/thrombin and selective thrombin inhibitors with significant oral bioavailability is described. This was achieved through minor modifications to the sulfonamide group in our potent and selective fXa inhibitor (*E*)-2-(5-chlorothien-2-yl)-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(morpholin-4-yl)-2-oxoethyl]-2-oxopyrrolidin-3-yl}ethenesulfonamide and these observed activity changes have been rationalised using structural studies.

Factor Xa (fXa) and thrombin are key enzymes in the coagulation cascade, and, in the search for orally active anticoagulants with improved efficacy/safety profiles, selective inhibitors of these enzymes are being extensively investigated.[1] As trypsin-like serine proteases, these enzymes exhibit a preference for basic groups to bind in their primary specificity (S1) pockets. First generation inhibitors incorporated a highly basic P1 group (e.g., benzamidine) to facilitate binding to Asp189 at the base of the pocket and whilst such compounds can possess high affinity, they generally suffer from poor oral pharmacokinetic profiles.

We recently reported a novel non-basic series of *N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}sulfonamides with different P1 groups as orally active fXa inhibitors.[2] This series exploits an alternative interaction in S1, specifically that between an aryl chloride and Tyr228,[3] and the (*E*)-2-(5-chlorothien-2-yl)ethenyl analogue **1** was identified as a candidate for further evaluation.[2b]

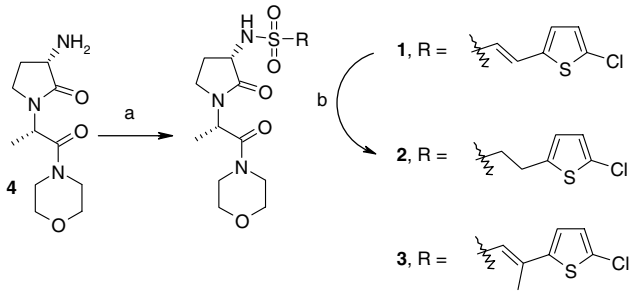
Compound **1** is a potent fXa inhibitor with good selectivity (90-fold) against thrombin, and shows both good anticoagulant activity and promising oral pharmacokinetic profiles in both the rat and the dog.[2b] Interestingly and unexpectedly, while investigating close structural P1 analogues of **1**, we have now found that the corresponding saturated analogue **2** is a potent, selective thrombin inhibitor, whereas the corresponding 5-(chlorothien-2-yl)prop-1-enyl analogue **3** is a potent dual inhibitor of fXa and thrombin.[4] We report here the initial biological profiles of these novel compounds. Furthermore, from X-ray structural information on their binding modes in fXa and thrombin we discuss insights gained of the factors governing their different enzyme inhibitory profiles.

Compounds **2** and **3** were synthesized as shown in Scheme 1. The key intermediate amine **4**, prepared from *N*-CBZ-L-methionine by the previously described five-step procedure,[2b] was readily converted into the secondary sulfonamides **1**–**3** by sulfonylation with appropriate sulfonyl chlorides, RSO_2Cl. Alternatively, **2** could be derived from **1** by catalytic hydrogenation.

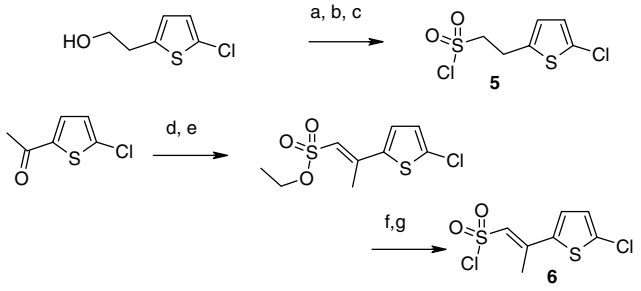
(*E*)-2-(5-Chlorothien-2-yl)ethenesulfonyl chloride was prepared by the literature route,[3a] whilst sulfonyl chlorides **5** and **6** were generated as shown in Scheme 2.[5a] 2-(5-Chlorothien-2-yl)ethanesulfonyl chloride **5** was readily derived from 2-(5-chlorothienyl)ethanol[5b] by conversion into the corresponding bromide and sequential

Keywords: Factor Xa; Thrombin; Oral; Dual inhibitor.
* Corresponding author. Tel.: +44 1438 768372; fax: +44 1438 763620; e-mail: Rob.J.Young@gsk.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.03.080



Scheme 1. Reagents: (a) RSO_2Cl, pyridine, MeCN; (b) $(Ph_3P)_3RhCl(I)$, H_2, AcOH.



Scheme 2. Reagents and conditions: (a) Ph_3P, CBr_4, 5 °C, THF; (b) Na_2SO_3, CH_3COCH_3, H_2O, reflux; (c) $POCl_3$, 150 °C; (d) CH_3SO_3Et, $[(CH_3)_3Si]_2NLi$, THF, −78 °C; (e) CH_3SO_3H, DCM, 0 °C; (f) *n*-Bu_4NI, CH_3COCH_3, reflux;[6a] (g) $POCl_3$, rt.[6b]

reaction with sodium sulfite and phosphorus oxychloride. (1*E*)-2-(5-Chlorothien-2-yl)prop-1-ene-1-sulfonyl chloride **6** was prepared from 2-acetyl-5-chlorothiophene by reaction with the lithiated anion of ethyl methanesulfonate followed by acid-catalysed dehydration of the derived alcohol and conversion of the resultant prop-1-ene-1-sulfonate ester into **6** via an adaptation of literature methods.[6]

In contrast to the potent, selective fXa inhibitor **1**, the saturated analogue **2** is a selective thrombin inhibitor whereas the related (*E*)-2-(5-chlorothien-2-yl)prop-1-enyl derivative **3** is a potent dual inhibitor of these enzymes. All three compounds possess good plasma-based activities in both the PT and aPTT assays (Table 1) and excellent selectivities against other trypsin-like serine proteases tested (Table 2).

We have reported previously that **1** showed encouraging pharmacokinetic profiles in rat and dog with high oral bioavailabilities in both species.[2b] Pharmacokinetic studies with **2** and **3** also revealed encouraging profiles

Table 1. fXa and thrombin inhibitory activities[7] and anticoagulant potency[8] for compounds **1**–**3**

Compound	fXa K_i (nM)	Thr K_i (nM)	1.5× PT (μM)	1.5× aPTT (μM)
1	4	367	1.2	1.3
2	154	17	2.6	1.6
3	2	2	0.54	0.32

Table 2. Selectivity profiles of compounds **1**–**3** expressed as IC_{50} values (nM) against other trypsin-like serine proteases[a]

Compound	aPC	Plas	Tryp	Kall	tPA
1	>20,000	>158,000	>158,000	15,800	>25,100
2	>25,100	>25,100	>25,100	>25,100	>25,100
3	>25,100	20,000	>25,100	5010	5010

[a] IC_{50} values (nM) from fluorogenic assays for all compounds. aPC, activated protein C; Plas, Plasmin; Tryp, Trypsin; Kall, Kallikrein; tPA, tissue plasminogen activator.

for these analogues with good oral bioavailabilities in these species (Tables 3 and 4).

These profiles made **2** and **3** attractive starting points for programmes of work directed towards novel series of orally active thrombin or dual thrombin/fXa inhibitors, respectively. Indeed, whilst other series of dual thrombin/fXa inhibitors have recently been described,[4] the profile of the novel, non-basic, inhibitor **3** is the first example of a potent dual inhibitor exhibiting similar potencies against each enzyme coupled with promising oral pharmacokinetic profiles in preclinical species.

Structural studies were undertaken with these compounds to provide insight into the basis of the highly interesting differences in enzyme activities and to facilitate a rational programme of structure-based design to exploit these fascinating results.

An X-ray crystal structure of **1** bound into fXa confirmed the expected binding mode for this class of inhibitors[2b] with the molecule unambiguously fitted to the $F_o - F_c$ electron density map.[10] Thus, the chlorothienyl group is

Table 3. Pharmacokinetic parameters of **1**–**3** in male Sprague–Dawley rats following intravenous and oral administration[9]

Compound	$t_{1/2}$[a] (h)	Clp[b] (ml/min/kg)	V_{ss}[c] (L/kg)	F[d] (%)
1	0.7	8	0.3	75
2	0.3	28	0.8	31
3	0.3	21	0.4	32

[a] $t_{1/2}$, half-life of the test compound expressed in hours.
[b] Clp, plasma clearance of the test compound expressed as mL/min/kg.
[c] V_{ss}, steady state volume of distribution of test compound expressed as L/kg.
[d] F, oral bioavailability of test compound expressed as percentage.

Table 4. Pharmacokinetic parameters of **1**–**3** in female[e] Beagle dogs following intravenous and oral administration[9]

Compound	$t_{1/2}$[a] (h)	Clp[b] (ml/min/kg)	V_{ss}[c] (L/kg)	F[d] (%)
1	1.2	4.6	0.4	53
2	0.5	13	0.5	38
3	0.6	5.7	0.3	48

[a] $t_{1/2}$, half-life of the test compound expressed in hours.
[b] Clp, plasma clearance of the test compound expressed as mL/min/kg.
[c] V_{ss}, steady state volume of distribution of test compound expressed as L/kg.
[d] F, oral bioavailability of test compound expressed as percentage.
[e] Data on compound **1** were generated in male dogs.

located in S1 where the chlorine atom makes the key interaction with Tyr228. The pyrrolidinone carbonyl group makes a conserved water-mediated interaction with Ser214 and the morpholine–alanylamide group fits into the S4 pocket formed by Phe174, Tyr99 and Trp215 (Fig. 1).

Similarly, an X-ray crystal structure of **1** complexed with thrombin revealed similar binding interactions for the P1 group and the carbonyl group of the central template although the sulfonamide is in a slightly altered position. In contrast, the P4 group adopts a different orientation and the morpholine ring occupies the S2 pocket formed by the 60's loop and S3 is unoccupied (Fig. 2). The S3 pocket, whilst lined by different residues, is available for binding so this different binding mode was unanticipated.[11] Significantly, the pendant alanine-derived methyl was orientated towards the S3 pocket, which provided a clear impetus for exploring larger substituents likely to enhance thrombin activity.[5b]

To determine whether this new binding mode occurred for all three compounds in thrombin the complex structures of **2** and **3** were determined.[11] The structures showed that all three compounds bind to thrombin with an identical binding mode to **1** (Fig. 3a). The pairwise RMSD between **1** and the other two inhibitors (conserved atoms) bound in thrombin having superposed the protein is 0.2 Å (**2**) and 0.3 Å (**3**) with a maximum distance between equivalent atoms of only 0.4 Å (for one of the carbon atoms in the morpholine ring). Since the binding modes are identical it seemed plausible that, the difference in thrombin inhibitory activity for the three compounds could be due to the conformational preference of the 5-chlorothienyl-based sulfonamides. Ab initio calculations using model systems for **1**, **2** and **3** have clearly indicated that this is the case.[11]

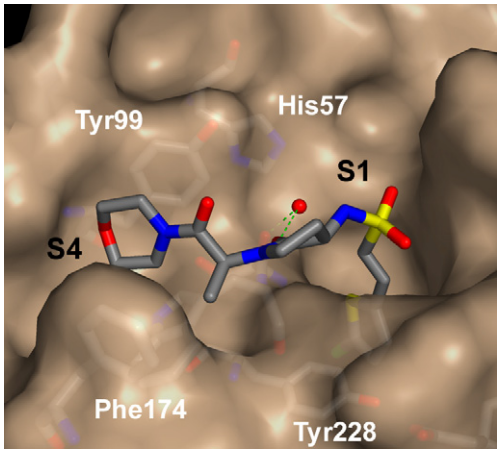


Figure 1. X-ray crystal structure of **1** complexed with fXa.[10]

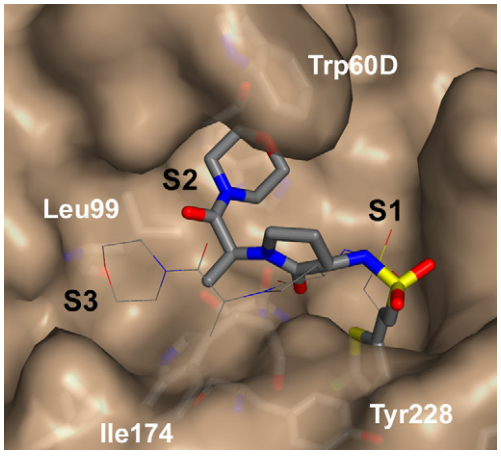


Figure 2. X-ray crystal structure of **1** complexed with thrombin.[11] Compound **1** bound to fXa (protein superposed) is shown in thin lines for reference.

It was noted that the position of the sulfonamide is different in the complex structures of **1** in fXa and thrombin (Fig. 2). This difference in position results in a different conformation of the 5-chlorothienyl-ethenyl group in fXa compared to thrombin. The results from the previously mentioned ab initio calculations[11] can be applied to this different conformation of **1** in fXa where the torsion angle (C═C)–(S═O) is approximately 110°. Assuming the binding mode for all three compounds remains the same, **1** and **3** are predicted to have energetically favourable conformations whilst there would be a significant energy penalty for the bioactive conformation of **2**. This might be expected to result in fXa inhibitory activities for **1** and **3** to be improved compared to **2** and this is in fact what is observed (Table 1).

This analysis is based on the assumption that the compounds maintain the same binding modes in fXa. The complex structures of **2** and **3** were undertaken to determine whether this was in fact the case. **2** was observed to bind to fXa in the same binding mode as **1**. RMSD calculated between **1** and **2** with protein atoms superposed is 0.4 Å. The binding mode of **3** in fXa, however, was seen to be different to that of **1** and **2** at the sulfonamide. Thus, whilst the chlorine atom interacts with Tyr228, and the central pyrrolidinone template and morpholine–alanylamide group make interactions analogous to those seen in the complex of **1** with fXa, the sulfonamide

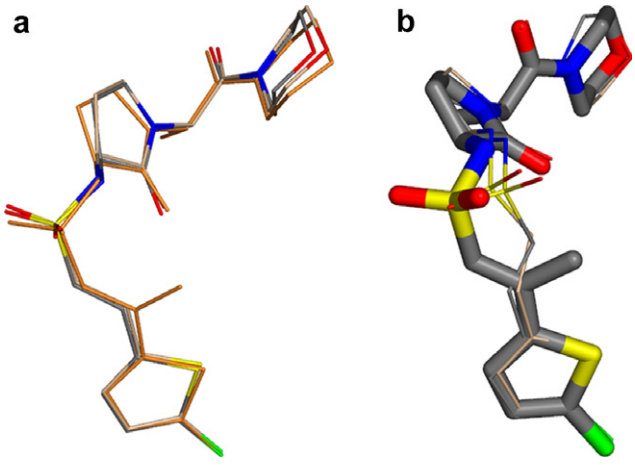


Figure 3. Superposition of protein bound molecules. (a) Superposition of **1** (grey), **2** (brown) and **3** (orange) in thrombin. (b) Superposition of **1** (thin grey), **2** (brown) and **3** (thick grey) in fXa.

adopts a conformation similar to that observed for all three compounds in thrombin (Fig. 3). As was seen for thrombin this alternate conformation is also energetically favourable. The additional atom in this molecule compared to **1** and **2** also results in additional non-specific interactions in the S1 pocket.

In summary, close analogues of the potent selective fXa inhibitor **1** have been identified as a potent and selective thrombin inhibitor **2** and potent dual inhibitor of fXa and thrombin **3**; both show excellent plasma-based activities and promising oral pharmacokinetic profiles in both the rat and the dog. The binding modes of all three compounds in fXa and thrombin have been unambiguously determined from X-ray crystallographic studies and these have provided insights into factors affecting their different selectivity profiles. The further evaluation of **2** and **3** and exploitation of these findings will be reported in future publications.

References and notes

1. (a) Steinmetzer, T.; Hauptmann, J.; Sturzbecher, J. *Exp. Opin. Invest. Drugs* **2001**, *10*, 845; (b) Quan, M. L.; Smallheer, J. M. *Curr. Opin. Drug Disc. Dev.* **2004**, *7*, 460; (c) Gould, W. R.; Leadley, R. *J. Curr. Pharm. Des.* **2003**, *9*, 2337; (d) Golino, P.; Loffredo, F.; Riegler, L.; Renzullo, E.; Cocchia, R. *Curr. Opin. Invest. Drugs* **2005**, *6*, 298; (e) Saiah, E.; Soares, C. *Curr. Top. Med. Chem.* **2005**, *5*, 1677; (f) Scarborough, R. M.; Pandey, A.; Zhang, X. *Ann. Rep. Med. Chem.* **2005**, *40*, 85.
2. (a) Watson, N. S.; Brown, D.; Campbell, M.; Chan, C.; Chaudry, L.; Convery, M. A.; Fenwick, R.; Hamblin, J. N.; Haslam, C.; Kelly, H. A.; King, N. P.; Kurtis, C. L.; Leach, A. R.; Manchee, G. R.; Mason, A. M.; Mitchell, C.; Patel, C.; Patel, V. K.; Senger, S.; Shah, G. P.; Weston, H. E.; Whitworth, C.; Young, R. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3784; (b) Chan, C.; Borthwick, A. D.; Brown, D.; Campbell, M.; Chaudry, L.; Chung, C.-W.; Convery, M. A.; Hamblin, J. N.; Johnstone, L.; Kelly, H. A.; Kleanthous, S.; Kurtis, C. L.; Patikis, A.; Patel, C.; Pateman, A. J.; Senger, S.; Shah, G. P.; Toomey, J. R.; Watson, N. S.; Weston, H. E.; Whitworth, C.; Young, R. J.; Zhou, P. *J. Med. Chem.* **2007**, *50*, 1546; (c) Senger, S.; Convery, M. A.; Chan, C.; Watson, N. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5731.
3. Similar findings have been reported for other series of fXa inhibitors (a) Choi-Sledeski, Y. M.; Kearney, R.; Poli, G.; Pauls, H.; Gardner, C.; Gong, Y.; Becker, M.; Davis, R.; Spada, A.; Liang, G.; Chu, V.; Brown, K.; Collussi, D.; Leadley, R., Jr.; Rebello, S.; Moxey, P.; Morgan, S.; Bentley, R.; Kasiewski, C.; Maignan, S.; Guilloteau, J.-P.; Mikol, V. *J. Med. Chem.* **2003**, *46*, 681; (b) Roehrig, S.; Straub, A.; Pohlmann, J.; Lampe, T.; Pernerstorfer, J.; Schlemmer, K.-H.; Reinemer, P.; Perzborn, E. *J. Med. Chem.* **2005**, *48*, 5900; (c) Jia, Z. J.; Su, T.; Zuckett, J. F.; Wu, Y.; Goldman, E. A.; Li, W.; Zhang, P.; Clizbe, L. A.; Song, Y.; Bauer, S. M.; Huang, W.; Woolfrey, J.; Sinha, U.; Arfsten, A. E.; Hutchaleelaha, A.; Hollenbach, S. J.; Lambing, J. L.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2073.
4. Other series of dual fXa/thrombin inhibitors have been reported (a) Deng, J. Z.; McMasters, D. R.; Rabbat, P. M. A.; Williams, P. D.; Coburn, C. A.; Yan, Y.; Kuo, L. C.; Lewis, S. D.; Lucas, B. J.; Krueger, J. A.; Strulovici, B.; Vacca, J. P.; Lyle, T. A.; Burgey, C. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4411; (b) Nar, H.; Bauer, M.; Schmid, A.; Stassen, J.-M.; Wienen, W.; Priepke, H. W. M.; Kauffmann, I. K.; Ries, U. W.; Hauel, N. H. *Structure* **2001**, *9*, 29; (c) Kranjc, A.; Kikelj, D. *Curr. Med. Chem.* **2004**, *11*, 2535.
5. (a) (*E*)-2-(5-chlorothien-2-yl)ethenesulfonyl chloride was prepared according to the procedure described in Ref. 3a.; (b) Borthwick, A. D.; Chan, C.; Kelly, H. A.; Peace, S.; Senger, S.; Shah, G. P.; Smith, S. A.; Smith, S.; Watson, N. S.; West, R. I.; Young, R. J. WO 04/052851 A1 2004.
6. The dehydration reaction gave a ca. 1:1 mixture of the required intermediate ethyl sulfonate ester and isomeric ethyl 2-(5-chlorothien-2-yl)-2-propene-1-sulfonate, which were readily separable by flash silica chromatography. (a) Gennari, C.; Longari, C.; Ressel, S.; Salom, B.; Meilgo, A. *Eur. J. Org. Chem.* **1998**, 945; (b) Abbenante, G.; Prager, R. H. *Aust. J. Chem.* **1992**, *45*, 1801.
7. fXa inhibitory activities were determined using Rhodamine 110, bis-(CBZ-glycylglycyl-L-arginine amide) as the fluorogenic substrate; as described in Ref. 2b; standard deviations: **1**, 1 nM; **2**, 49 nM; **3**, 1 nM. Thrombin inhibitory activities were similarly determined using rhodamine 110, bis-(CBZ-L-valyl-L-prolyl-L-arginine amide) as described in Ref. 5b; standard deviations: **1**, 22 nM; **2**, 3 nM, **3**, 1 nM. Standard deviations are derived from at least $n \geqslant 2$ experiments for both assays.
8. Anticoagulant activities were determined in the prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays; details are described in Refs. 2b and 5b. Standard deviations in PT assay: **1**, 0.04 μM; **2**, 0.39 μM; **3**, 0.04 μM. Standard deviations in aPTT assay: **1**, 0.01 μM; **2**, 0.22 μM; **3**, 0.04 μM. Standard deviations are derived from at least $n \geqslant 2$ experiments for both assays.
9. The formulation used for both iv and po dosing to rat and dog was a 5:95% (v/v) mixture of DMSO and 50:50 PEG-200/sterile water. For all animal studies, serial blood samples were collected into heparinised containers at various time-points and blood centrifuged to yield plasma. These studies used three animals for each (iv/po) leg, except $n = 2$ rat data for **2** and **3**.
10. The complexes for **1**, **2** and **3** with fXa showed clear density for the ligand in initial $F_o - F_c$ difference maps. The structures were refined at 1.9, 1.75 and 1.75 Å, respectively, to a final R_{factor}/R_{free} of 0.19/0.24 (**1**), 0.18/0.21 (**2**) and 0.20/0.26 (**3**), using procedures described in Ref. 2b. Co-ordinates are deposited in the protein data bank with codes of 2uwl, 2uwp, 2uwo.
11. Senger, S.; Chan, C.; Convery, M. A.; Shah, G. P.; Hubbard, J. A.; Watson, N. S.; Young, R. J. *Bioorg. Med. Chem. Lett.* **2007**, *17,* doi:10.1016/j.bmcl.2007.02.034.